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Ethylenebisdithiocarbamates and Ethylenethiourea: Possible Human Health Hazards

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Dithiocarbamates are widely used as fungicides because of their efficacy against a broad spectrum of fungi and their associated plant diseases. Dithiocarbamates are also used in industry as slimicides in water-cooling systems, in sugar, pulp, and paper manufacturing, and as vulcanization accelerators and antioxidants in rubber. Because of their chelating properties, they are also used as scavengers in waste-water treatment.

Dithiocarbamates can be divided into two groups: the ethylenebisdithiocarbamates (EBDC), such as maneb, zineb, and mancozeb, and the dimethyldithiocarbamates (DMDC), including ferbam, ziram, and thiram. In recent years there has been increasing awareness of the hazards of various synthetic organic chemicals to living organisms.

Ethylenethiourea (ETU) is one of the principal metabolites of EBDCs and is thought to be the source of most of the toxicity associated with EBDCs. ETU is a degradation by-product of the manufacture of EBDCs (in tobacco, cooked foods, commercial beverages, etc.), formed during product storage (1–4). ETU is also the major identifiable product of ultraviolet irradiation, according to Gruiskshan and Jarro, who studied its photolysis and hydrolysis (5). EBDCs are metabolized by mammals to ETU (6,7). Extensive methylation of ETU occurs in the cat, but not the rat (8). Although no studies of ETU formation have been conducted in humans, ETU has been found in the urine of potato farmers exposed to EBDCs (9), implying that such metabolism occurs.

Because ETU can be produced by hydrolytic thermal decomposition of EBDCs, it is difficult to analyze. Several rather cumbersome techniques that have been used and the best currently available technique are reviewed below (10–13).

Even though the acute toxicity of pure EBDCs is low, the health of workers may be jeopardized by inhaling ETU or EBDCs (14). ETU is hydrosoluble and has raised concern because of its thyroid toxicity and tumorigenicity (15–18). In this review, we summarize and analyze the available data on EBDCs used as pesticides to indicate their impact on mammals.

Presence of ETU in Commercial Food Products

Several foods purchased locally were found to contain small quantities of ETU (10,19). Pease and Holt (20) reported that tomatoes (including tomato juice, soup, canned tomatoes, and catsup), potatoes, cucumbers, summer squash, and cantaloupes taken from 17 different locations throughout the United States where maneb was applied according to label directions showed no residue of ETU (<0.05 ppm), even in the presence of 4 ppm of maneb (1 day after the last of three applications on tomatoes).

Wine contained 0.037 ppm ETU and no residue of EBDC. ETU residues in beer ranged from 0.026 to 0.07 ppm (21). Studies with [¹⁴C]ETU show that more than 80% of the applied dose remained in the beer after fermentation (19).

ETU is produced by burning cigarettes, at a quantity of about 50% of the EBDC residue in the tobacco (determined by measurement of carbon disulfide). For instance, 32 ppm of EBDC in tobacco produced about 16 µg ETU/g, of which one-tenth was absorbed by the smokers (19). Such direct absorption by smokers, and absorption by nonsmokers through passive smoking, along with absorption from consumption of cooked vegetables containing EBDC residues, may be the most important source of exposure to ETU among the general public (19,22).

Kinetics and Metabolism

In general, dithiocarbamates can be absorbed via the skin, the mucous membranes, and the respiratory and gastrointestinal (GI) tracts. Whereas dithiocarbamates are absorbed rapidly from the GI tract, metal-complexed alkylene bisdithiocarbamates are absorbed poorly from both the GI tract and through the skin (23).

ETU is rapidly absorbed from the GI tract and cleared from the body in all mammalian species that have been tested. After only 5 min, ETU appeared in the blood of rats administered an oral dose of 100 mg/kg body weight of [¹⁴C]ETU. Within 48 hr, 82–99% of an oral dose was eliminated via

Humans are exposed to ethylenebisdithiocarbamates (EBDCs) from environmental sources. Exposure to EBDCs is chronic for workers in a variety of industries, where EBDCs are used for their properties as slimicides, vulcanization accelerators, antioxidants, and scavengers in waste-water treatment. EBDCs, and particularly the EBDC metabolite ethylenethiourea, have clearly defined, important toxic effects in various animal species, and there is reason to suspect they are carcinogenic in humans. In the absence of definitive information regarding human risk, further studies need to be done. In the interim, regular surveillance of workers with high levels of exposure to EBDCs, with specific attention to markers of thyroid and hepatic pathology, should be considered. **Key words:** dithiocarbamates, ethylenebisdithiocarbamates, ethylenethiourea, hepatocellular carcinoma, thyroid cancer, zineb. *Environ Health Perspect* 103: 568–573 (1995)

the urine and about 3% via the feces (24,25). Newsome (6) and Ruddick et al. (26) found that approximately 70% of ETU was eliminated in the urine and 1% in the feces; comparable results were found for mice, while in monkeys 55% was eliminated via the urine within 48 hr and less than 1.5% via the feces (27).

ETU and its metabolites have a half-life of about 28 hr in monkeys, 9–10 hr in rats, and 5 hr in mice (25). In a study of the accumulation and elimination of radioactivity by the thyroid gland of rats dosed with ETU, dose levels of 2 and 200 µg [¹⁴C]-labeled ETU were administered daily for 14 days (28). In a second experiment rats were dosed with 0, 0.1, 1, 10, 50, or 100 mg [¹⁴C]ETU/kg body weight in food daily for 7 days (28). The first experiment showed that the concentration of ETU and/or its metabolites in the thyroid is dose dependent, and the second experiment showed that the level of [¹⁴C] in the thyroid did not increase appreciably when the daily dose was increased above 50 mg/kg diet. Withdrawal of ETU from the diet led to an 80–94% reduction in the radioactivity in the thyroid after 17 days (28).

In a recent study conducted in potato growers, ETU, used as a biological indicator of EBDC exposure, had a long half-life (32–100 hr) and could therefore be detected in urine even several days after exposure (9). Other investigators (29) reported that

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in a sample of 10 farmers exposed to dithiocarbamate, monitored throughout the entire exposure period (approximately 5 hr), ETU was identifiable in urine samples collected immediately after exposure in only three, and persistence of ETU in urine over the entire 40-hr period after exposure was observed in only one subject. However, Kurttio and Savolainen (9) reported an elimination half-life for ETU of close to 100 hr in 38 potato farmers exposed to EBDCs.

The metabolic decomposition of EBDCs in mammals is complex and results in the formation of carbon disulfide, ethylene diamine, a few ethylene bistiuram disulfides, ethylene bistiocyanate, hydrogen sulfide, and ETU. Dithiocarbamates and their metabolic products are found in certain organs, such as the liver, kidneys, and especially the thyroid, but accumulation of these compounds does not take place because of their rapid metabolism (23).

With regard to the hepatic metabolic system, liver monooxygenases in rats are inhibited by oral administration of ETU (30). In mice, however, these same researchers found opposite effects: ETU treatment caused an increase in cytochrome P450 content and aniline hydroxylase activity. In addition, a dose 20 times that producing inhibition of aminopyrine *N*-demethylase activity in rats had no effect on this enzyme in liver microsomes of mice.

Lewerenz and Plass (31) suggested that the different response of hepatic microsomal enzymes may contribute to the difference in acute toxicity and teratogenicity between mice and rats. This implies that ETU is likely metabolized by different enzymatic pathways. Compounds identified after oral administration of ETU in the urine of mice were 2-imidazolin-2-yl-sulfenates, ethylene urea, and unchanged ETU (32–34). The *S*-oxidation of ETU occurred in the microsomal fraction of mouse liver (32). Imidazole, ethylene urea, imidazolone, and unchanged ETU were detected in the urine of rats (8).

Hui (35) noted that although flavin-dependent monooxygenase metabolism and binding is a major pathway for ETU biotransformation in the mouse, the toxicological significance of this fact is not clear. Siddiqui et al. (36) comparatively evaluated the acute effects of the EBDC fungicides mancozeb and zineb on microsomal mixed-function oxidase (MFO) in male albino rats. The differences in the inhibitory effectiveness of mancozeb and zineb toward MFO may be attributed to the presence of both Mn^{2+} and Zn^{2+} in mancozeb and zineb molecules, differences in their absorption and disposition kinetics, and/or in their metabolic products. In addition, the tendency of these fungicides

to chelate metal-containing enzymes such as cytochromes (37) appears to be another likely mechanism of their toxicity. Mn^{2+} (38) and Zn^{2+} (39) have been shown to elicit a significant inhibition of hepatic microsomal hydroxylation of aniline and *N*-demethylation of aminopyrine. It has been suggested that this inhibition of monooxygenase occurs as a result of denaturation of cytochrome P450 brought about by an interaction of EBDC with the sulfhydryl groups of cytochrome P450 and by some other unknown mechanism, probably related to its lipophilic character.

Analytical Methods

Since EBDC tolerances for zineb were first established in 1955, the official methodology used by the U.S. Food and Drug Administration (FDA) for analyzing EBDC residues on food has been a non-specific method based on the colorimetric determination of a yellow complex formed by reaction of evolved carbon disulfide (CS_2) with a reagent after acid hydrolysis of the sample (40). Because this method cannot distinguish between residues of individual EBDCs, all EBDC tolerances are based on zineb.

A further limitation of this method is that it cannot distinguish EBDC residues from those of another major subgroup of dithiocarbamate fungicides, the dimethyldithiocarbamates (ferbam and ziram). Today, refinements of this basic methodology can separate and measure residues of individual EBDCs on crops.

The analytical methodology for ETU has been more actively developed because of concern about its presence in food. The FDA methodology for residues does not detect ETU, since ETU cannot evolve CS_2 . However, several reports have described analytical methods for ETU (1,2,12,41–46).

Gas chromatography is still the major technique for the residue analysis of ETU, using precolumn derivatization to achieve sensitivity and specificity (2,6,47,48). Under suitable clean-up and GC conditions, the procedures allow a detection limit of 10 ppb in various foodstuffs. The derivatization procedures are, however, time consuming, and additional ETU may be formed during reactions at elevated temperatures as a result of decomposition of EBDC residue present in the sample.

Column liquid chromatography (LC) has also been applied using either nonselective UV absorbance detection with a rather complicated sample clean-up (11,49), or selective electrochemical detection, for which sample clean-up is much simpler (46,50,51). This method can be done with much simpler sample clean-up. In fact, for determination of ETU in wine and beer,

down to a level of about 50 ppb, no clean-up is necessary at all (52).

These GC and LC methods share the disadvantages of laboriousness and/or insufficient sensitivity. Hogendoorn et al. (13) therefore investigated the potential of reverse-phase LC column switching for trace-level determination of ETU in water samples. They found that column switching was adequate for the clean-up and concentration of chloroallyl alcohol (CAAL) from aqueous samples, even in combination with relatively nonselective detection (UV, 205 nm). Because ETU is considerably more polar than CAAL and is consequently even less retained on a C_{18} column, development of a reverse-phase column switching procedure is inherently more difficult. This study reports such a development for the trace-level determination of ETU in ground waste samples down to about 0.05 ppb.

Other analytical methods of determining ETU have also been reported, including thin-layer chromatography (TLC), polarography, and radioisotope dilution. A variety of absorbents and developing solvents (TLC) have been used to detect ETU in plants (1,12,53,43). Semi-quantitative determinations are possible by comparison with ETU standards run simultaneously (54). Polarography involves clean-up on an alumina column, followed by paper chromatography and determination of the nitroso derivative by polarography (1). Radioisotope dilution, a reverse isotope dilution method, has been used to determine ETU in the presence of its metabolites and is useful in the low milligram range (41,54).

Animal Studies

ETU generally has low acute toxicity, but it does induce a wide spectrum of anomalies in many test animals fed significant doses for various lengths of time. The most prominent aspect of ETU toxicology is its action on the thyroid gland, with resultant hyperplasia and decrease in thyroid hormone levels. ETU has also been shown to have teratogenic, carcinogenic, immunotoxic, and mutagenic effects in various animals studies (55–58).

Carcinogenicity. Several studies have shown that the prolonged administration of ETU to rats causes thyroid neoplasms (17,41,56,59–61). Ulland (17) included 350 and 175 ppm ETU in the diet of male and female rats, which led to a dose-related induction of follicular and papillary thyroid cancers, and of related lesions such as thyroid solid-cell adenomas and hyperplastic and simple goiter. The first tumor was found after 68 weeks, and most cancers occurred after 18–24 months, when the study was terminated.

Recently Chhabra et al. (61) studied the perinatal and adult exposure of ETU induction of thyroid neoplasms in rats and identified similar thyroid effects in mice. They confirmed that ETU was carcinogenic in both male and female rats at dietary concentrations of 83 and 250 ppm, regardless of the level of perinatal exposure. Males were more sensitive than females, as demonstrated by a higher incidence of follicular cell neoplasms (adenoma or adenocarcinoma).

The possible mechanism of thyroid follicular cell carcinogenesis in rodents has been reviewed recently (62–65). Exposure to ETU, for either 9 months or 2 years, led to a general decrease in serum T_4 levels and increase in serum thyroid-stimulating hormone levels in both rats and mice. These hormonal changes correlated with morphological changes in the thyroid glands, suggesting that ETU carcinogenesis may be due to an imbalance of thyroid–pituitary homeostasis.

Chronic ETU administration produces hepatocellular carcinoma in the mouse and rat (16,56,61). Innes (16) reported induction of liver neoplasms in mice exposed to dietary concentrations of 646 ppm ETU for 18 months. Two strains of mice fed ETU in the diet had an increased incidence of hepatomas (56). Centrilobular hepatocellular cytomegaly occurred in male and female mice and rats receiving ETU at concentrations of 500 ppm or greater. Hepatocytes surrounding the central venules were enlarged, with homogeneously staining, finely granular eosinophilic cytoplasm (61).

Thus, toxicological studies have generally shown that although the thyroid is the major site of ETU-induced carcinogenicity in rats (17,59,60), the liver is also a major target of ETU-induced carcinogenicity in mice (16).

While the incidence of neoplasms and non-neoplastic lesions after exposure to EBDCs or ETU is only clearly proven to be increased in the thyroid gland or liver, several isolated studies suggest a possible effect on other sites as well. Chhabra et al. (61) showed statistically significant increases in neoplasms of the Zymbal gland and of the hematopoietic system in ETU-treated rats, as compared to control animals. There was also a statistically insignificant trend toward a slight increase in rare renal tubular cell neoplasms. In addition, Chernov and Khistenko (66) and Balin (67) have reported that zineb and maneb induced pulmonary adenomas in mice after oral administration. Finally, Ulland et al. (17) described pulmonary metastases in rats with oral exposures.

Mutagenicity. With regard to the mutagenicity of EBDC, Seiler (68) found

that maneb was negative in tests with *Salmonella* strains HisG46, TA1530, TA1531, and TA1532, and doubtful in TA1534. In addition, maneb and zineb given intraperitoneally to mice at 100 mg/kg body weight caused chromatid aberrations in bone marrow cells (55,69).

For ETU, tests with a large number of *S. typhimurium* strains gave mostly negative results (70,71). However Seiler (72) reported weak (dose unrelated) mutagenicity in *S. typhimurium* strain G46. ETU was also mutagenic in a host-mediated assay of *S. typhimurium* TA1530, when mice were dosed with 6000 mg ETU/kg body weight, but not at doses of 2000 mg/kg or less (73). Schüpbach and Hummler (73,74) also reported that ETU appeared to induce base-pair mutations but not frameshift mutations in *S. typhimurium* TA98, TA1537, and TA1538 exposed to ETU in the presence of dimethylsulfoxide and/or liver microsomes (25).

Teratogenicity. EBDCs are teratogenic in rats, mice, and hamsters, but not in cats. In rats, the major reproduction indices were unaffected by maneb dietary levels of ≤ 250 mg/kg diet. There was no histological evidence of congenital anomalies in a variety of tissues and organs of male and female rats of the F_{3b} litter subjected to histopathological examination (75). Maneb, zineb, and mancozeb exert dose-dependent damaging effects on the gonads of rats of both sexes. The dose levels used were 96–960 mg/kg body weight of zineb, 140–1400 mg/kg of mancozeb, and 14–700 mg/kg of maneb, each given twice a week for 4.5 months. Both reproductive and endocrine structures were affected at all dose levels, leading to decreased fertility (76,77). In a 4-month inhalation study on rats using maneb at 4.7 mg/m^3 , no effect on sperm motility was detected (78). Administration of 100 mg/kg body weight of zineb to rats for a period of 2, 4, or 6 months produced delayed insemination, sterility, resorption of fetuses, and developmental anomalies (79).

In both rats and hamsters exposed to EBDCs, malformations of the brain predominate (80,81). The major teratologic effect is the development of hydrocephalus and other central nervous system defects postnatally, resulting in a high mortality rate among the offspring (56). In studies by Larsson et al. (82), maneb was administered to Sprague-Dawley rats at dose levels of 0, 400, 770, or 1420 mg/kg body weight, by gavage, as a single dose on day 11 of gestation. Gross malformations occurred in all surviving animals at 770 and 1420 mg/kg, but no malformations were observed in the single litter of the low-dose group. CDI mice administered 0, 375, 750, or 1500 mg maneb/kg body

weight on days 7–16 of gestation showed a decrease in fetal caudal ossification centers at all dose levels (83). Abnormalities included cleft palate, hydrocephalus, and other serious defects. Abnormalities found at the highest dose level may have been due in part to the presence of ETU in the formulation (84).

In contrast to these findings in other species, teratogenicity has not been observed in cats given daily doses of 0, 5, 10, 30, 60, or 120 mg ETU/kg (85). The explanation for this exception is not certain, but it may be that extensive methylation of ETU occurs in the cat, but not the rat (8).

Human Studies

The acute toxicity of dithiocarbamates is low, and acute intoxication in human beings is therefore unlikely. There is a report, however, of a 62-year-old man who developed acute renal insufficiency after applying maneb. A causal relationship of the exposure is not clear, however, because the patient had a history of hypertension, cerebral infarction, and gastrectomy and chemotherapy (for stomach cancer). The patient was treated with hemodialysis and survived to hospital discharge (86). Temporary alterations in central nervous functions (87), diarrhea, and acute renal and transient heart failure (86) have also been reported after acute exposure to EBDCs.

In numerous studies EBDCs and ETU have been strong skin sensitizers (88–93). The irritant and allergic potential of most dithiocarbamates is evident upon occupational exposure.

Cases of diffuse erythema and eczematoid epidermatitis of the eyelids and inguinal regions, probably with elements of sun sensitization, were observed among agricultural workers (grapes and tobacco) in contact with zineb (94) or maneb (95–98).

In a rarely cited epidemiologic study, which is the only such study currently available, von Meyer (99) found trends toward increasing incidences of human liver and thyroid cancers in several geographic areas of the United States, in relation to the degree of exposure to dithiocarbamates, as determined by sales and crop production statistics for dithiocarbamate-containing pesticides. The study was conducted in four states (New York, Florida, Maine, and Pennsylvania), with an overall sample size of more than 27 million people in the countries surveyed. In comparison to very low baseline national incidences of these cancers, the slight increases in the exposed communities (over a 20-year period, following introduction of widespread agriculture use of these pesticides) were for the most part not statistically significant.

In 1978, Lybarger (100), in a study of workers at a plant that produces ETU in the United States, found only slight differences in levels of T_4 and T_3 in 43 workers and 7 controls subjects. Smith (101) carried out a study of thyroid function which was somewhat limited by the small number of workers and by the intermittent nature of the mixing work. He found no evidence that thyroid function is severely affected by exposure to ETU at the levels experienced by these workers, nor was there evidence of any clinical effect. Only one worker was considered hypothyroid upon biological testing, although T_4 results in the exposed workers were generally somewhat lower than those in control subjects.

A Parkinsonian syndrome has also been reported in two young, previously healthy agricultural workers after chronic exposure to maneb (102). Subsequent evaluation of 50 other workers similarly exposed on a chronic basis found significantly increased incidences of various neurologic effects (including cogwheel rigidity, fatigue, and complaints of memory loss) and increases in a variety of other Parkinson-like symptoms (including tremor, ataxia, and bradykinesia) compared to a control group of workers not exposed to this pesticide. Although such toxic effects are probably attributable to the manganese contained in this pesticide, the possibility of an independent contribution of the EBDC itself cannot be entirely eliminated.

Discussion

Human exposure to EBDCs, and the toxic EBDC metabolite ETU, is not insignificant. Although these compounds affect primarily workers in specific industries, ETU is also present in relatively small quantities in a number of common foodstuffs. It is therefore important to analyze the risks incurred by such exposures, particularly in light of the known toxicities of these chemicals in animals.

The toxicokinetics of EBDCs have been well studied. EBDCs are absorbed slowly through the GI tract and skin and are metabolized via hepatic microsomal enzymes to produce ETU, which appears to be the major cause of toxicity of these chemicals. ETU is rapidly absorbed via the GI tract (23). The elimination of ETU is largely renal (27), and its elimination half-life is variable, ranging between 32–100 hr, according to the species involved (9,25). EBDC residues accumulate preferentially in the thyroid, and secondarily in the liver (28).

Differences in acute toxicity as well as in teratogenicity are seen in mice and rats exposed to ETU directly. In addition, teratogenicity does not occur in the cat, which unlike other species extensively

metabolizes ETU to its *S*-methyl derivative. Thus, interspecies differences in hepatic microsomal enzyme activity, regarding both the formation of ETU from EBDCs and its subsequent transformation, are most likely responsible for the variable toxicity in different species (8,31–33). Since these same metabolic pathways for EBDC and ETU may also be different in humans, there may also be major differences in the toxicity of EBDCs in humans, in ways that cannot be easily predicted.

It is possible today to chemically evaluate the presence of EBDCs and ETU with a fairly high degree of accuracy. Although many methods initially proposed in the literature are neither adequately sensitive or specific, a method described by Hogendorn in 1991 (13), based on a reverse-phase LC column-switching technique, has proven to be quite specific and sensitive enough to allow detection of residual quantities on the order of parts per trillion.

It is clear that EBDCs and ETU are potentially toxic in chronically exposed animals. Although it remains difficult to evaluate the effects of acute intoxications with these agents because only a few studies of such toxicity have been done, the toxicity of prolonged exposures is definitive. Adverse effects include a variety of acute symptoms, as well as evidence of carcinogenesis, mutagenesis, and teratogenesis. Although several authors have described adverse effects on the liver (16,56,61), and one investigator has actually stated that the liver is the primary target organ (60), the bulk of evidence suggests that it is in fact the thyroid which is most adversely affected by these chemicals (17,41,56,59–61). Thyroid and hepatic neoplasms both occur in animals exposed to EBDCs and ETU, but the teratogenicity of ETU appears to be limited to rats and hamsters (8,26,33,71,80), among species thus far studied. The major target organ of this teratogenicity, it is generally agreed, is the brain (56,80,81).

Since the different toxic response seen among various animal species is best explained by differences in their metabolism of EBDCs, such that one cannot easily extrapolate these findings directly to humans, and since few studies of exposure to EBDCs have been conducted in humans, our current knowledge of the subject must be considered quite limited. Nevertheless, there is enough evidence to conclude that these chemicals exert at least some toxic effect, the maximum degree of which is not yet certain.

EBDCs and ETU definitely have an irritant effect on human skin (88–93), but information regarding more important types of toxicity is inconclusive. With regard to pathologic changes in the thyroid

and the liver, no direct relation has been established vis-a-vis exposure to EBDCs, although one large epidemiologic study may suggest a possible relationship between use of dithiocarbamates and human liver and thyroid cancers. In this study there were statistically insignificant trends toward increased incidence of these neoplasms in four geographic areas of the United States where dithiocarbamate-containing pesticides are most commonly used, as evidenced by sales and crop production statistics (99). Although von Meyer downplays the significance of the small differences noted, it remains possible that excess cancer rates were not proven statistically only because of insufficient power of the study, despite its size and 20-year duration. In the presence of extremely low baseline rates of these cancers in the general community, and given the diluting effect of surveillance of all individuals in a geographic area, including many who are not exposed to the putative causative agent, it would require enormous sample sizes to find even relative increases in cancer incidence. Because no other studies have been done on the subject, the question of possible carcinogenicity of EBDCs in humans remains unanswered.

Conclusions

Humans are exposed to EBDCs, especially certain classes of workers for whom this exposure is chronic. Such EBDCs, and particularly the EBDC metabolite ETU, have clear and important toxic effects in various animal species, and there is reason to at least suspect possible carcinogenicity of these agents in humans. It is therefore desirable that further human studies be done to better define and assess this risk. Such research is feasible today, in light of available methods to quantitate degrees of exposure to these chemicals. Furthermore, pending the results of such studies, it would clearly be desirable to undertake regular surveillance of humans exposed to EBDCs, with specific attention to markers of thyroid and hepatic pathology.

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